STUDIES ON BACTERIOLOGICAL BIOLUMINESCENCE, ACTION OF MAGNESIUM SALT

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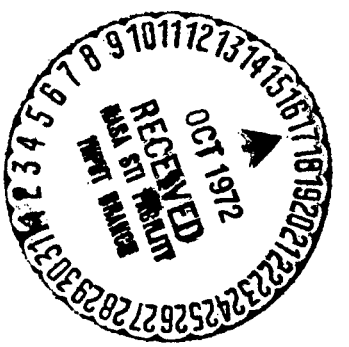
Translation of an article in Bolletino delle Societe di Naturalist, Vol. 33, 1920, pp. 112-119.



NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 JUNE 1972

Source: Bulletino delle Societe di Naturalisti, Vol 33, 1920, pp 112 - 119
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Studies on Bacteriological Bioluminescence.

Action of Magnesium Salt

[redacted] by Dr. Giuseppe Zirpolo

It is well known that some magneisum salts (carbonate and sulphate) are used in various soil cultures for the development of bacteria. The solution of Cohn, the three solutions of Naegeli, those of Mayer, Winogradsky, Ouchinsky, Raulin, d'Arnaud and Charin, Maassen, Iacsch, the first and the third of Proskauer and Bech contain a percentage that varies from gr. 0.02 per mille to 1.00.

We know, furthermore, that marine waters contain the sulphates and chloride of magnesium in the proportion of 3.239 gr. per mille for the first and of 2.196 gr. per mille for the others, according to the data of Forschammer, and, moreover, from the analyses made by Schloesing in the Mediterranean waters, giving a percentage of magnesium of 2.365 gr. per mille at a temperature of 20°C.

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Such considerations have caused me to deal first with the action of the magnesium sulphate on phosphorescent bacteria and of various other salts of the same metal, to see in what proportion each of these would produce the greatest activity, not only on their intensity, but also in the duration of the luminosity.

My research was almost completed when I was able to read about some work by Shoij on the action of the salt of calcium, litium, ammonium, potassium, sodium, rubidium, cesium, magnesium, barium, stroncium, manganese, iron and zinc on the luminous organs of a cefalopodium; the Watasenia scintillans Berry. The author has found that among the various salts that were used, those of magnesium (chloride and sulphate) produced the most favorable action on the luminosity of the organs that were studied.

My research was initiated simultaneously with that of Shoij. While my research confirmed what had been found by that author, it also generalized it in that I focused on magnesium, studying which of the various salts of this metal was most favorable to the development and duration of the bacteriological luminosity. Furthermore, I performed experiments on another bacteriological type whose morphological, cultural, and pathogenetical characteristics had been exhaustively studied by myself in former research, and this type maintained the tubes luminous for about five months, while Shoij had obtained a maximum light duration of a few hours given by the luminous organs of the Watasenia scintillans Berry.

Lastly, we must remember that Harvey, who had performed well-known studies on animal luminousity, had also delt with the action of some organic and inorganic substances on the luminousity of bacteria.

His research, though worthy, was not performed on a determined species of bacteria, and, furthermore, his observations were recorded only after ten minutes, one hour, and twenty-four hours. Such rapid observations do not allow certain deductions, because, as it is well known, and we shall see this on the following pages, that luminousity appears sometimes according to the concentration of the salts only after two or three days.

The findings of Harvey that $MgCl_2$ is toxic has, for the moment, no value.

PERSONAL RESEARCH

Topic for Study and Methodology

In this research, I have used the bacillus Pierantonii Zirpolo. The culture of this bacterium was made in broth of sepia consisting of seawater, pepton 1% and alkalinized with sodium carbonate. In various tubes we introduced a determined quantity of the mixture so as to obtain dilutions from 1% to 25% of the various magnesium salts used. These were 1) magnesium salicylate, 2) neutral citrate of magnesium 3) magnesium chloride, 4) magnesium sulphate 5) magnesium tartrate.

When the dilutions were made and the broth sterilized, a drop of well measured culture of luminous bacillus Pierantonii Zirpolo of twenty-four hours was introduced with a small sterilized pipe into each of the tubes.

Our purpose was to have placed more or less the same number of bacteria in equal quantities of solutions, with varying proportions of salts, to see not only how long the development of bacteria took in the single tubes, but also what the intensity and duration of their luminousity was. To these we added control tubes with no magnesium salt.

During the whole time of the experiment the tubes remained in a ground floor room which was completely dark with an average temperature of 15°C. Observations were made every day at first and successively in periods of several days.

Action of the Various Salts of Magnesium on the Development, Intensity, and Duration of the Light of the Bacillus Pierantonii Zirpolo

I - Salicylate of Magnesium

This salt proved to be completely detrimental for photobacteria. In the proportion that we used from 1% to 25%, there was not even a trace of bacteria development. This salt, therefore, exercises a deleterial action on luminous bacteria and must be put aside for this research.

On the other hand, it is well known that salicylate acid is antiseptic.

2. Neutral Magnesium Citrate

This salt has a favorable action on the development of phosphorescent bacteria, only in the proportion of 1%. In the successive dilutions, from 2% to 25%, did I have no development of light. The light in the tube with the proportion of 1% increased in intensity during the first four days after being placed in the tube; it reached a maximum at the seventh day and then decreased until there was no trace after the forty-fifth day.

The surface of the liquid presented a film which gave a strong green light after five days, but thereafter, during the following days, the film cracked. All the other tubes which contained the same salt in a proportion of 2% to 25%, not only gave no light, but did not produce a film either; this fact indicated to us that the magnesium citrate salt is detrimental to the life of the photobacteria, beginning with a proportion of 2% and upwards.

3. Magnesium Chloride

The first night we started the experiment with the luminous culture, I noticed, after a few minutes, a great luminosity in the tubes containing salt in the proportions of 1% to 15%; in the other tubes, containing from 16% to 25%, I saw no light that night nor in the following nights.

The following day the light became very powerful in the tubes con-

taining 1% to 3% magnesium chloride, and this light continued to increase greatly on the following day, thereafter returning to normal and dimming.

After the fifth day, the light appeared in the tube with the proportion of 7%, but soon after became dim and remained thus for the next 32 days. In the tubes with 1% proportion, the light remained for a period of four months, and in the tube with a 5% proportion, this occurrence lasted for about two and a half months.

4. Magnesium Sulphate

This salt produced a luminosity which lasted for quite a long time. Just a few minutes after the experiment was begun, the tubes containing magnesium sulphate of from 1% to 25% were phosphorescent.

The following night the tubes from 1% to 3% proportion showed more luminosity, and less light was produced in those which contained a proportion of from 24% to 25%.

The following night the light increased and stayed very strong during 11 days in the tubes containing magnesium sulphate in the proportion of from 1% to 13%.

After approximately two months, the light given out by the control tube was very inferior to that produced by the tube containing salt in the proportion up to 1%. The light in the tubes containing magnesium sulphate

in the proportion from 1% to 21% lasted for a period of 45 days and in the tubes containing this salt in the proportion of 1% to 6% the light lasted for some additional time.

After the third day, we had the film formation, which gave out a very bright light which had a beautiful intense green color. This salt is found to be much better than all the other salts, examined up to now, due to the fact that it produces a very bright light, particularly a few nights after the beginning of an experiment, and preserves this light for a very long time. In addition, this light is far brighter than the light emitted from the control tubes without magnesium sulphate.

5. Magnesium Tartrate

This salt is the best among all that we used, because it allows the development of very bright light of long duration in all the concentrations we made.

In the tubes with the concentration varying from 1% to 25%, the light was splendid, and remained bright for 10 days, reaching the maximum of its intensity on the fifth day.

In the tubes having concentration of 1% the light intensity decreased but remained bright for approximately twenty-five (25) days and the light was much brighter than that shown by the control tubes.

In the other tubes having a concentration from 2% to 25%, the light was dim for more than five (5) months, but could still be easily noticed. Also in these tubes we had a formation of a film that gave a bright green light that could be seen from several yards.

As you can see from this research, the magnesium cation has a favorable action on the development of the bacteriological luminescence; only the various anions used have different influences on the development of the light and precisely in the following sequence:

Tartrate ≫ Sulphate ≫ Chloride ≈ Citrate ≈ Salicylate

We can see from what I was saying in the preceeding paragraph and from the results of my observations the great importance of the magnesium salts - except Salicylate - on the development of phosphorescent bacteria on the length of their light. Now we just have to investigate the origins of the property of the magnesium on the development and the duration of the light of the photobacteria.

I think that the recent studies of Wilstaetter on chlorophyll can help us in this research, in fact this author, after a long series of experiences, was able to demonstrate that the magnesium is tied to the complex of the organic molecule and has an important action on the organic functions.

From these studies it is easy to understand how the photobacteria,

developing in a solution of various concentration of magnesium salts, could use these salts according to their needs, giving not only a very bright green light that could be observed also during the day time, but also a light having an intensity that was never obtained.

After the research of Willstaetter, magnesium salts have been largely used in experiments undertaken to study their action on the development of the trees, (Mameli, Bernardi L., Morelli, M.). This research is very interesting and, as we expected, gave very good results.

We can say that the action of magnesium ought to be favorable to the development of the photobacteria; in fact, being in a solution very rich of this metal, in a salt state, they were able to assimilate it.

We must not think that the photobacteria could assimilate a larger quantity of magnesium salt dissolved in the broth. (We can, for instance, remember that if we feed animals with iron salts the hemoglobin does not assimilate more of what it requires).

But, that being in a solution where life conditions are more favorable, they can have a remarkable and continuous development. This fact could explain that the greater luminousity is due to the greater number of bacteria developed and that the longer duration of light is due to their prolonged activity during their development.

CONCLUSIONS

1. The various magnesium salts used, except the magnesium salicylate, extraordinarily activate not only the intensity of the light in the phosphorescent bacteria, but also its duration.

2. Among all these salts we used the one which has less power on the luminescence is the neuter magnesium citrate and following progressively magnesium chloride, the sulphate and the Tartrate:

Tartrate ➢ Sulphate ➢ Chloride ➢ Citrate ➢ Salicylate

3. The luminescence bacteria can live well in various solutions of magnesium salts and, for a longer time, in solutions containing salts in concentrations up to 11%.

4. The magnesium tartrate is the most favorable to the development and to the duration of the light of the photogenic bacteria. The light shown by bacteria living in solutions with proportions of this salt from 1% to 23% has been really exceptional and the light remained quite brilliant for a longer period of time as compared with all the others.

5. The various concentrations of salts do not tell us that the bacteria can assimilate more quantity of magnesium than they require, but being in solutions where life is more favorable they can develop more rapidly.

This fact would also explain the brighter luminousity; the longer duration of light should be due to the abundance of nourishment given to them.

Naples, Zoological Station, November, 1919.

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